Thermal-Fluidic System for the Manipulation of Biomolecules and Viruses

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We are developing a reconfigurable fluidic system that demonstrates the ability to *simultaneously* perform separations, concentrations, and purifications of biomolecules and viruses using temperature gradient focusing (TGF). Many projects throughout LLNL, particularly those related to pathogen detection, mitigation, and protection, require the manipulation of biomolecules or viruses to accurately 1) quantify the presence of a particular substance; or 2) synthesize and investigate the function of a molecule.

This novel microfluidic technology is an equilibrium gradient version of capillary electrophoresis (CE) that allows for the stationary fractionation and concentration (up to 10,000 x) of target analytes on the dimension of bulk or free solution electrophoretic mobility. In this technique a delicate balance is achieved in a microchannel between a net fluid flow and an opposing electrophoretic velocity gradient to capture charged analytes at a specific location (Fig. 1). The analytes are then separated based on their free solution electrophoretic mobility.

Project Goals

The project goals are to develop an automated TGF instrument to improve the separation resolution and throughput when applied to front-end sample processing of biological samples. Two specific application areas are identified to demonstrate the novel sample manipulation capabilities inherent to TGF: 1) the purification and separation of different virus strains in complex samples; and 2) improving the performance of protein concentration and separation for *in vitro* transcription/translation (IVT) protein expressions.

Relevance to LLNL Mission

TGF specifically addresses LLNL's interests in the detection of biomolecules, viruses, or cells at low concentrations through enhanced collection, separation, and purification strategies. This is facilitated by performing the necessary front-end sample preparation through concentration procedures and removing noisy background signals/contaminants. This project supplies LLNL engineers with a novel capability to perform biomolecular, viral, and cellular control in a flexible format to address a wide range of programmatic assay conditions.

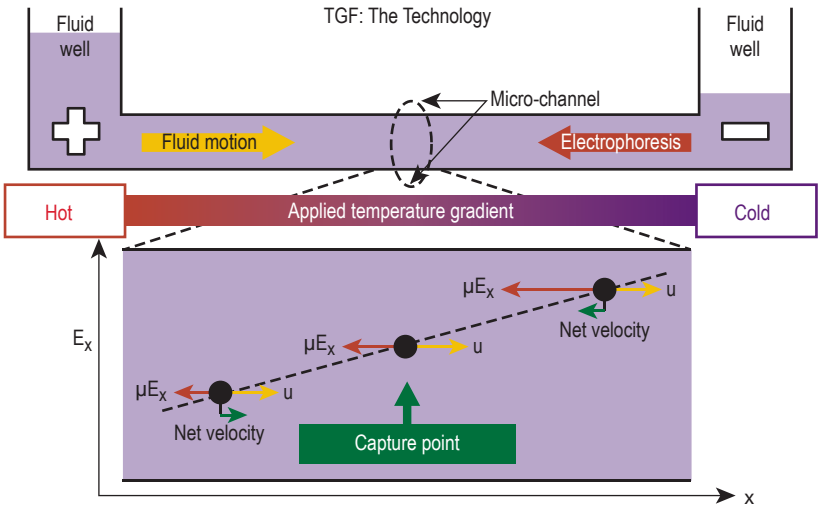

Figure 1. Schematic of temperature gradient focusing (TGF). Bulk fluid motion (yellow arrow) is balanced by an opposing electrophoretic velocity (red arrow) to capture analytes at a unique spatial location within a specific electrophoretic mobility range.

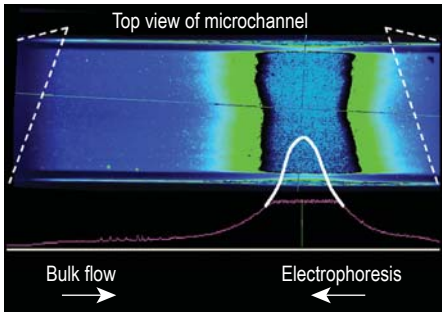

Figure 2. Automated TGF capture and concentration (>2000-fold) of a fluorescent dye.

FY2007 Accomplishments and Results

Automated TGF capture. We demonstrated the successful capture and concentration (> 2000-fold) of a small fluorescent dye molecule in an automated sequential injection analysis system (Fig. 2). We improved the overall instrument performance by improving the stability of the applied electric field and flow rate, improving the linearity of the temperature field, and increasing the magnitude of the temperature gradient.

Stable flow. We designed a pressure-controlled flow system with integrated flow rate feedback to achieve the desired ~1 nl/min flow rate control. Our flow rate control system enables steps, ramps, and other functions (Fig. 3) necessary for our project goals.

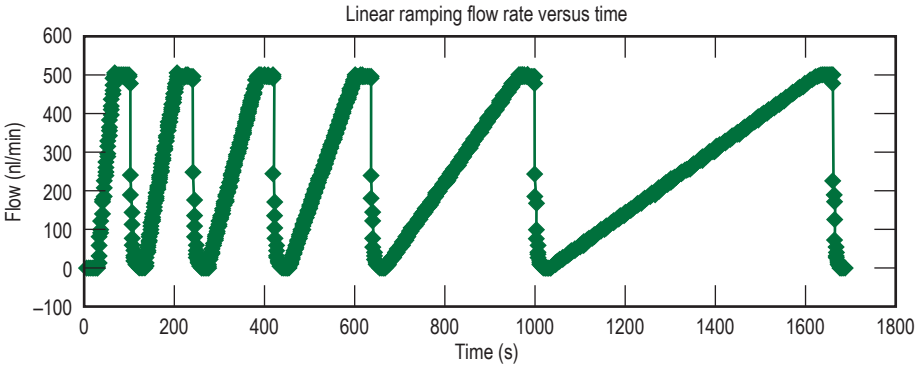

Figure 3. Flow rate stability of LLNL pump control system in a varying flow rate ramping mode.

Linear temperature gradients. We used numerical modeling of the governing equations (energy equation and Stokes flow) and multi-physics simulations (COMSOL Multiphysics) to determine optimal device designs for the generation of thermal gradients (50 ˚C/mm) in a high-throughput microfluidic structure. An example of the temperature gradient as a function of applied electric field is shown in Fig. 4. Our models demonstrate the need for "thick walled" capillaries and robust thermal mounting to the heat source. We achieve these requirements using microfabricated glass chips with etched microchannels in a copper package using compression mounting and a high performance thermal interface material.

Modeling effort. For the initial modeling of the relevant field variables (temperature, velocity, and voltage) we use the commercially available finite element modeling package, COMSOL Multiphysics. To solve for the analyte concentration profiles in a TGF system, these known field variables feed into a Brownian dynamics simulation. From there specific metrics, such as peak height, peak width, throughput, and limit of detection, are extracted to determine the efficiency and resolving power of a particular TGF run (Fig. 5). Design guidelines and rules (geometric and operating conditions) were constructed for various applications to aid in the design and testing of TGF devices.

FY2008 Proposed Work

In FY2008, we plan to 1) move from fluorescent analytes to biological samples (virus and protein); 2) build up a fluorescently labeled sample library (virus and protein) to characterize system performance with biological samples; 3) organize the library into biologically relevant groupings; 4) determine how biologically relevant groupings of our sample library relate to electrophoretic mobility; 5) demonstrate an ability to separate three spiked viral samples in a "simplified" background (repeat with proteins) and purify spiked virus from a "dirty" background of multiple contaminants (repeat with proteins); and 6) move to more relevant and complex sample matrixes (nasopharyngeal and IVT protein productions).

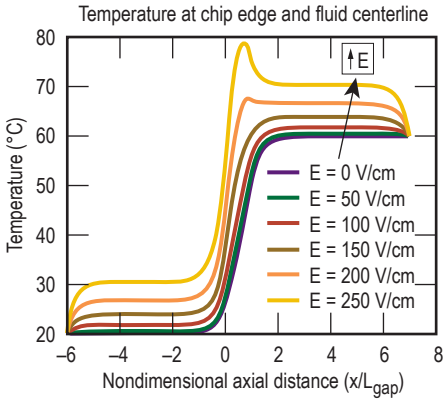

Figure 4. Numerical simulation results showing the varying temperature profile in the focusing region of our microfluidic chip. The model includes externally applied temperature gradients, natural convective losses through the packaging, nonuniform Joule heating, and convective/conductive conjugate heat transfer through the fluid/wall structure.

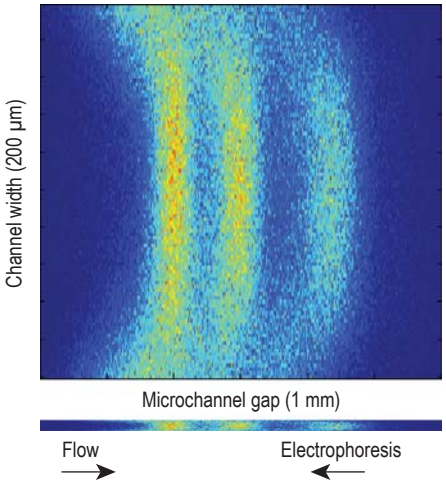

Figure 5. Monte Carlo particle tracking simulations, predicting the steady state shape and band location of three analytes with different electrophoretic mobilities that are captured, concentrated, and separated using TGF. The top image is scaled to aid in visualization.